REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
02-05-2016	Final	Feb2011-Jun 2014

4. TITLE AND SUBTITLE

Intraosseous hydroxocobalamin versus intravenous hydroxocobalamin compared to intraosseous whole blood or no treatment for hemorrhagic shock in a swine model

5a. CONTRACT NUMBER

5b. GRANT NUMBER

FWH20110148A

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Bebarta, Vikhyat S.
Garrett, Normalynn
Boudreau, Susan M.
Castaneda, Maria G.

5d. PROJECT NUMBER

FWH20110148A

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

59th Medical Operations Squadron
Wilford Hall Ambulatory Surgical Center
2200 Bergquist Drive Bldg 4550
Lackland AFB, TX 78236

8. PERFORMING ORGANIZATION REPORT NUMBER

FWH20110148A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of the Air Force Surgeon General
Directorate for Modernization, SGRS
5201 Leesburg Pike, Suite 1206
Falls Church, VA 2240

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution A. Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Objective: Determine if intraosseous (IO) hydroxocobalamin (HOC) can improve systolic blood pressure (SBP) after severe hemorrhagic shock. Method: 36 swine (45-55kg) anesthetized, intubated with continuous monitoring. Controlled hemorrhage of 30% of blood volume over 20 min. Randomized to receive 500mL IO blood, 150mg/kg IO or intravenous (IV) HOC in 180mL saline or no treatment. Monitored for 60 min post treatment. Sample size of 8 animals per group. Results: RMANOVA outcome analysis detected a significant difference between groups($p<0.05$). IO whole blood, IO HOC, and IV HOC were similar to each other but significantly different compared to controls regarding SBP, MAP, SVR and heart rate. Differences in SBP and MAP were sustained throughout the experiment. Conclusion: IO HOC improved SBP and MAP compared to no treatment and was similar to IO whole blood and IV HOC in this animal model of severe hemorrhage. Moreover, whereas serum lactate was improving in all treated groups, in was deteriorating in the control group.

15. SUBJECT TERMS

hydroxocobalamin, intraosseous, infusion, swine, hemorrhage, resuscitation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Vikhyat S. Bebarta, MD
U	U	U	SAR		19b. TELEPHONE NUMBER *(Include area code)* 210-727-2696

WHASC – Animal Final Report

30Apr2014

1. Protocol Number: FWH20110148A

2. Type of Research:
Animal Research

3. Title:
Cobalamin- A small volume, blood sparing, neuroprotective drug for hemorrhagic shock resuscitation in swine (Sus Scrofa).

4. Principal Investigator (PI):

Name	Rank	Date of IACUC Training	Branch of Service/ Corps	Staff Resident Fellow Civilian	Department / Office Symbol	Email (if other than WHASC Outlook)	Phone	Pager
Vikhyat Bebarta	O-5	09 Feb 2012	USAF	Staff	59 EMDS/SG OED	vikhyat.s.bebarta.mil@mail.mil	WP: 292-3908	Cell 275-3794

5. Purpose:
To determine if hydroxocobalamin, a portable, safe and FDA approved drug, is effective in improving hemorrhagic shock

6. Results:

Intravenous (IV) versus proximal tibial intraosseous (IO) hydroxocobalamin (HOC) compared to no treatment:

Systolic blood pressure, the primary outcome variable, was similar between the IV and IO HOC groups over time. This was significantly different from the non-treated group such that at 60 minutes, mean SBP for IV and IO HOC were 75.1 and 83.7 mm Hg, respectively, compared to 55.3 mm Hg for control. Similar to SBP, HR, and MAP significantly improved over time in both treated groups but not in the control group. By 60 minutes, mean HR was 116 and 135 bpm and MAP was 60 and 65 mm Hg for IV and IO HOC groups (not significantly different) whereas non-treated animals displayed a mean HR of 157 bpm and MAP of 43 mm Hg.

IO HOC produced a statistically significant increase in systemic vascular resistance (SVR) at 10 minutes compared to IV HOC and control (SVR 2805 vs. x 1526 vs. 1029 dyne-sec-cm^{-5}). Cardiac output was not significantly different among the groups over time (3.4 vs 3.2 vs 3.3 L/min).

RMANOVA modeling by time revealed a difference by group such that at 60 minutes serum lactate was significantly higher in the control group (mean 3.36 mmol/L) compared to the IV or IO groups (mean 1.36 and 1.63 mmol/L). However, PT and PTT were normal among the three groups suggesting hydroxocobalamin had no effect on coagulation. Mean PT and PTT for IV vs. IO vs. no treatment at 60 minutes were the following: PT 14.6 vs. 14.8 vs. 13.6 sec; PTT 33.8 vs. 34.0 vs. 33.2 sec.

Intravenous hydroxocobalamin versus intravenous whole blood (WB) compared to control:

At five minutes and fifteen minutes after hemorrhage, two animals in the whole blood group died, leaving 8 animals in the WB to complete the study. Data from the non-surviving animals were excluded from analysis. Systolic blood pressure, the primary outcome variable, was similar between the IV HOC and WB groups over time. This was significantly different from the non-treated group such that at 60 minutes, mean SBP for IV HOC and WB were 75.1(3.8) and 80.8(4.2) mm Hg, respectively, compared to 55.3(3.8) mm Hg for control. Similar to SBP, HR, and MAP significantly improved over time in both treated groups but not in the control group. By 60 minutes, mean HR was 116(9.9) and 100(11.2) bpm and MAP was 57(3.9) and 62(4.4) mm Hg for IV HOC and WB groups (not significantly different) whereas non-treated animals displayed a mean HR of 157(9.9) bpm and MAP of 43(3.9) mm Hg.

Animals in the IV HOC group demonstrated significantly greater systemic vascular resistance over time compared to non-treated animals but not compared to animals treated with WB (mean SVR at 60 minutes 1316(72.5) vs. x 882(72) vs. 973(81) vs. dyne-sec-cm^{-5}). In addition mean cardiac output (CO 3.4(0.3) vs.

For Protocol Office Use Only:
Received on:_____ Initials_____ Report Due Date_____
Scheduled for IACUC:_____ Copy to Budget? Yes No BIRDS Agenda
Who Signed? PI Co-PI Auth AI

3.3(0.27) vs. 4.6(0.3) L/min) was significantly lower in the IV HOC and non-treated animals compared to IV WB treated animals.

Evaluation of hydroxocobalamin compared to Hextend®:

There were no significant differences between the IV HOC and Hextend® groups at baseline or at shock (HR 94 vs. 81 bpm; SBP 47 vs. 51 mm Hg; MAP 39 vs. 43, mm Hg; SVR 790 vs. 949 dyne-sec-cm5; lactate 1.2 vs. 1.4 mmol/L). Post bleed the overall RMANOVA model detected no significant difference by time between groups ($p>0.05$). At 60 minutes cardiovascular parameters of HOC vs. Hextend® were the following: HR 116 vs 90 bpm; SBP 75 vs 85 mm Hg; and MAP 60 vs. 64 mm Hg. By 60 minutes, serum lactate levels were falling in both groups from mean peak at 20 minutes post bleed of 1.43 vs. 1.81 mmol/L to 1.36 vs. 1.45 mmol/L. Conclusion: A small volume of IV HOC improved blood pressure and reduced serum lactate as well as IV Hextend®.

Efficacy of introaosseous hydroxocobalamin versus intraosseous plasma compared to no treatment:

Repeated measures MANOVA detected a significant difference among groups ($p<0.00$). Variables showing a significant difference were measured with a Bonferroni adjustment. By 60 minutes, SBP, MAP, and SVR values were not significantly different between HOC (mean SBP 83; MAP 65 mm Hg; SVR 1538 dyne-sec-cm-5) and plasma groups (mean SBP 84; MAP 68 mm Hg; 1569 dyne-sec-cm-5) but significantly different from control (mean SBP 55 mm Hg; MAP 44 mm Hg; SVR 882 dyne-sec-cm-5) suggesting the HOC and plasma groups were recovering from shock, but not control. Although there was no statistically significant difference in serum lactate, by 60 minutes lactate for the HOC or plasma groups was near baseline (mean lactate IO HOC 1.7 mmol/L; plasma 1.6 mmol/L) whereas lactate was rising in the control group (mean lactate 3.4 mmol/L), supporting the cardiovascular data. Our data suggests that IO HOC is as effective as IO plasma in treating hemorrhage and significantly more effective compared to no treatment.

Comparison of intraosseous hydroxocobalamin to intraosseous whole blood:

SBP and MAP improved in the HOC and WB groups, such that by 60 minutes mean SBP was 83 and 78 mm Hg and MAP was 65 and 63 mm Hg. These parameters were not significantly different from each other, whereas they were different from the control group (mean SBP 55; MAP 44 mm Hg). Although there was no statistically significant difference or interaction among the groups with regard to serum lactate, by 60 minutes lactate was falling toward normal in the WB and HOC groups (mean lactate 1.05, 1.63 mmol/L), but rising in the control group (mean lactate 4.0 mmol/L). Conclusion: IO HOC compares favorably to WB in treatment of hemorrhagic shock and both improve blood pressure compared to no treatment.

7. How may your findings benefit the Air Force?

Hydroxocobalamin could be a useful adjunct to hemorrhagic shock in prehospital, tactical situations. It is a small volume, small weight, safe, FDA approved drug that also reduces inflammation and is neuroprotective.

8. Number of Animals

Projected Enrollment of Animals at the Beginning of Study: 78

Actual Number of Animals Enrolled: 93

9. Status of Animals Entered Into the Protocol: Two of the pigs (pig #5666 and 5665) were determined to be ill by Dr. Harroff and were replaced at that time, when allowed. Pig number 6411 died prior to the end of the monitoring period. Pig numbers 6492 and 6808 died during the hemorrhage portion of the experiment. Necropsies were ordered and the reports from the pathologist are attached. All animals were euthanized per protocol.

10. Status of Funds: All funds have been allocated. There was a 120 day delay in receiving the funds from AFMSA to CRD. We had no budget deviations. We were funded for 3 years by AFMSA.

11. Reason for Closure:

- Objectives of the study were met

12. Specific Problems: After receiving the NO monitor and working closely with a representative from Harvard Apparatus, we decided to postpone this portion of the research protocol, as it was not feasible to collect NO through this new device for our study.

13. Publications and Presentations:

Presentations:

Society for Academic Emergency Medicine (SAEM):
May 2013- Two oral presentations
May 2014- Two oral presentations and 2 posters

Medical Military Health Science and Research Symposium (MHSRS):
August 2014- Five posters

These Presentations and Publications have been cleared by 59 CRD and Public Affairs.

Publications:
None

These Presentations and Publications have/have not (choose one) been cleared by 59 CRD and Public Affairs. (Do not delete this sentence.)

14. Exceptional Achievements:

Society for Academic Emergency Medicine **(**SAEM**):**
Gallery of Excellence poster presenter May 2014

15. Signature of Principal Investigator:

_____
VIKHYAT BEBARTA, Lt Col, USAF, MC
Chief, Medical Toxicology
Department of Emergency Medicine – WHASC and SAMMC
vikhyat.s.bebarta.mil@mail.mil
275-3794 (Cell)

For Protocol Office Use Only:
Received on:_____ Initials_____ Report Due Date_____
Scheduled for IACUC:_____ Copy to Budget? Yes No BIRDS Agenda
Who Signed? PI Co-PI Auth AI

PATHOBIOLOGY, 59TH CLINICAL RESEARCH DIVISION, SGVUO

2200 Bergquist Drive, Bldg. 4430, Lackland AFB, Texas 78236-9908 Phone: (210) 292-6589/7190 Fax: (210) 292-6053 DSN: 554-6589/7190

ACCESSION NUMBER:	INVESTIGATOR: Dr. Bebarta	DEPARTMENT:	PROTOCOL NUMBER:
14-011	Telephone:	Emergency Medicine	FWH20110148A
PROSECTOR:	ANIMAL NUMBER:	SPECIES: Pig	SEX:
Thompson	6808	BREED:	F
DATE OF ARRIVAL AT CRD:	WEIGHT:	DATE OF DEATH:	EUTHANASIA METHOD:
19 Feb 14	115lbs	25 Feb 14	Died on Table
DATE OF BIOPSY:	DATE OF NECROPSY:	DATE TISSUE RCVD:	DATE OF REPORT:
N/A	25 Feb 14	25 Feb 14	4 Mar 14

CLINICAL HISTORY

Patient died during blood draw. Approximately half the amount of blood was removed and then the patient died.

Cassette 1= Lung
Cassette 2= Heart
Cassette 3= Heart, lymph node
Cassette 4= Liver, spleen
Cassette 5= Lung, lymph node
Cassette 6= Stomach, jejunum, adrenal gland
Cassette 7= Kidney
Cassette 8= Kidney

Tentative Clinical Diagnosis: R/O Pre-existing disease.

PATHOLOGIC SUMMARY

Gross Description:

Large amount of foam present in large airways. Caudal dorsal lung lobes are mottled pink and red. Multifocal white depressed areas ("milk spots") are present on the liver (R/O *Ascaris suum* larval migration).

Morphological Diagnoses:

1. Lung: Pneumonia, bronchointerstitial, eosinophilic, lymphoplasmacytic, histiocytic, and neutrophilic, multifocally extensive, marked, with edema and BALT hyperplasia.
2. Lymph node, inguinal and tracheobronchial: Lymphoid follicular hyperplasia, diffuse, moderate.
3. Lymph node, tracheobronchial: Draining hemorrhage, acute, multifocal, moderate.
4. Lymph node, inguinal and tracheobronchial: Draining eosinophils, multifocal, mild to moderate.
5. Liver: Hepatitis, periportal, eosinophilic, diffuse, mild to moderate, with fibrosis.
6. Heart: Myocarditis, interstitial, lymphoplasmacytic and eosinophilic, multifocal, mild.
7. Jejunum: Enteritis, eosinophilic and lymphoplasmacytic, diffuse, mild.
8. Kidney: Nephritis, interstitial, lymphoplasmacytic, multifocal, mild.
9. Stomach; spleen and adrenal gland: No significant findings.

Comments:

The most likely cause of this patient's death is respiratory compromise secondary to pneumonia coupled with anesthesia. The histomorphologic findings in the lung are consistent with a viral pneumonia superimposed with a bacterial infection. The pattern of lymphocytic inflammation in the lung (primarily around airways and blood vessels) is characteristic of *Mycoplasma hyopneumoniae*; however, Porcine Arterivirus (Porcine Reproductive and Respiratory Syndrome – PRRS), and Porcine Circovirus 2 are also differential diagnoses. Additionally, all three may be present concurrently and all three can cause primary lung disease and impair normal pulmonary defenses against inhaled bacterial pathogens. Establishing a definitive diagnosis in cases of swine pneumonia requires laboratory support (viral isolation/bacterial culture/IHC/PCR). The eosinophilic inflammation suggests an allergic or parasitic etiology as well – most likely secondary to *Ascaris suum* larval migration. Lymphoid follicular hyperplasia is indicative of chronic antigenic stimulation. The eosinophilic inflammation found in multiple sites is most likely secondary to *Ascaris suum* larval migration. The remaining findings are incidental.

Michelle E Thompson

MICHELLE E. THOMPSON
LTC, VC, USA
Chief, Pathobiology

Histology Use Only: Blocks 8 H&E Slides 8 Specials 0 Unstained 0 Grossed on: 26 Feb 14 by LTC Thompson Date Completed: 4 Mar 14

PATHOBIOLOGY, 59TH CLINICAL RESEARCH DIVISION, SGVUO

2200 Bergquist Drive, Bldg. 4430, Lackland AFB, Texas 78236-9908 Phone: (210) 292-6589/7190 Fax: (210) 292-6053 DSN: 554-6589/7190

ACCESSION NUMBER:	INVESTIGATOR: Dr. Bebarta	DEPARTMENT:	PROTOCOL NUMBER:
13-189	Telephone:	Emergency Medicine	FWH20110148A
PROSECTOR:	ANIMAL NUMBER:	SPECIES: Pig	SEX:
Thompson	6492	BREED:	F
DATE OF ARRIVAL AT CRD:	WEIGHT:	DATE OF DEATH:	EUTHANASIA METHOD:
15 Jul 13	50kg	25 Jul 13	Euth Solution
DATE OF BIOPSY:	DATE OF NECROPSY:	DATE TISSUE RCVD:	DATE OF REPORT:
N/A	25 Jul 13	25 Jul 13	5 Aug 13

CLINICAL HISTORY

Patient was part of hemorrhage study. Patient had a blood lactate of 1.6. Blood pressure looked good (116/75) and MAP 91 so proceeded with protocol. At 5 minutes post-bleed we gave 500 ml whole blood IV. Approximately 1 minute post-bleed, patient's pressure dropped and it died. The abdomen appeared distended upon arrival and throughout procedure.

Tentative Clinical Diagnosis: R/O Pre-existing disease

Cassette 1= Lung
Cassette 2= Lung
Cassette 3= Lung
Cassette 4= GI
Cassette 5= GI

PATHOLOGIC SUMMARY

Gross Description:
None provided.

Morphological Diagnoses:
1. Lung: Congestion, acute, diffuse, moderate.
2. Lung: BALT hyperplasia multifocal, mild to moderate with atelectasis.
3. Colon, GALT: Lymphoid follicular hyperplasia, diffuse, moderate, with suspect intrahistiocytic, basophilic, botryoid inclusion bodies.
4. Small intestine: No significant findings.

Comments:
The acute pulmonary congestion is likely a terminal event associated with acute cardiovascular collapse. Lymphoid follicular hyperplasia in multiple sites is indicative of chronic antigenic stimulation. There are suspect botryoid inclusion bodies in many of the macrophages present in the GALT which would be consistent with Porcine Circovirus 2 (PCV2). I am sending a block to the Michigan Diagnostic Center for Population and Animal Health for PCV2 immunohistochemistry. An addendum will follow.

Michelle E Thompson

MICHELLE E. THOMPSON
MAJ, VC, USA
Chief, Pathobiology

Histology Use Only: Blocks 5 H&E Slides 5 Specials 0 Unstained 0 Grossed on: 30 Jul 13 by MAJ Thompson Date Completed: 5 Aug 13